ABSTRACT

A method for the stepwise creation of phosphodiester bonds between desired nucleosides resulting in the synthesis of polynucleotides having a predetermined nucleotide sequence by preparing an initiation substrate containing a free and unmodified 3'-hydroxyl group; attaching a mononucleotide selected according to the order of the predetermined nucleotide sequence to the 3'-hydroxyl of the initiating substrate in a solution containing a catalytic amount of an enzyme capable of catalyzing the 5' to 3' phosphodiester linkage of the 5'-phosphate of the mononucleotide to the 3'-hydroxyl of the initiating substrate, wherein the mononucleotide contains a protected 3'-hydroxyl group, whereby the protected mononucleotide is covalently linked to the initiating substrate and further additions are hindered by the 3'-hydroxyl protecting group. Methods in which a mononucleotide immobilized on a solid support is added to a free polynucleotide chain are also disclosed.